

**FINAL REPORT**

**Test Facility Study No. 511873**

**Primary Skin Irritation/Corrosion Study with  
MLA-3202  
in the Rabbit  
(4-Hour Semi-Occlusive Application)**

**SPONSOR:**

Chemtura Corporation  
199 Benson Road  
MIDDLEBURY, CT 06749  
USA

**TEST FACILITY:**

Charles River Laboratories Den Bosch B.V.  
Hambakenwetering 7  
5231 DD 's-Hertogenbosch  
The Netherlands

**29 August 2016**

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## 1. STATEMENT OF GLP COMPLIANCE

Charles River Den Bosch, 's-Hertogenbosch, The Netherlands

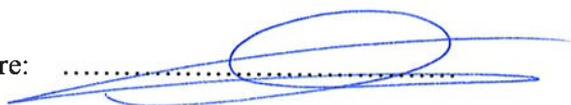
All phases of this study performed by the test facility were conducted in compliance with the following GLP regulations:

- OECD Principles of Good Laboratory Practice concerning Mutual Acceptance of Data in the Assessment of Chemicals, 26 November 1997 (C(97) 186 Final);
- EC Council Directive 2004 (2004/10/EC, February 11, 2004, Official Journal of February 20, 2004).

The data generated and reported are considered to be valid.

Charles River Den Bosch

Signature: .....



Name: A.H.B.M. van Huygevoort, MSc.

Title: Study Director

Date: .....

..29 August 2016

**2. TEST FACILITY QUALITY ASSURANCE STATEMENT**

Charles River Den Bosch, 's-Hertogenbosch, The Netherlands.

Study title: Primary skin irritation/corrosion study with MLA-3202 in the rabbit (4-hour semi-occlusive application)

This report was inspected by the Charles River Den Bosch Quality Assurance Unit (QAU) according to the Standard Operating Procedure(s). The reported method and procedures were found to describe those used and the report reflects the raw data. During the on-site process inspections, procedures applicable to this type of study were inspected. The dates of Quality Assurance inspections are given below.

**Project** 511873

Type of Inspections	Phase/Process	Start Inspection date	End Inspection date	Reporting date
<b>Study</b>	Study Plan	08-Jun-2016	08-Jun-2016	08-Jun-2016
	Report	09-Aug-2016	09-Aug-2016	09-Aug-2016
<b>Process</b>	<b>Test Substance Receipt</b> Test Substance Handling	09-May-2016	20-May-2016	24-May-2016
	<b>Test Substance Formulation</b> Test Substance Handling	30-May-2016	13-Jun-2016	14-Jun-2016
	<b>Animal Facilities</b> Test Substance Handling Exposure Observations/Measurements Specimen Handling	04-Jul-2016	15-Jul-2016	22-Jul-2016

The review of the final report was completed on the date of signing this QA statement.

The facility inspection program is conducted in accordance with Standard Operating Procedure.

Charles River Den Bosch

Signature:  .....

Name: C. Mitchell B.Sc., FRQA  
Head of Quality Assurance

Date: 26 Aug 2016 .....

### 3. SUMMARY

Primary skin irritation/corrosion study with MLA-3202 in the rabbit (4-hour semi-occlusive application).

The study was carried out based on the guidelines described in:  
OECD No.404, "Acute Dermal Irritation/Corrosion" (2015)  
EC, No 440/2008; B4: "Acute Toxicity: Dermal Irritation/Corrosion".  
US EPA, OPPTS 870.2500 (1998), Acute Dermal Irritation.  
JMAFF Guidelines (2000), including the most recent revisions.

Three rabbits were exposed to 0.5 mL of MLA-3202 by application onto clipped skin for 4 hours using a semi-occlusive dressing. Skin reactions were assessed 1, 24, 48 and 72 hours and 7 and 14 days after exposure for the first animal and 1, 24, 48 and 72 hours after exposure for the other two animals.

Exposure to MLA-3202 resulted in very slight to well-defined erythema and very slight oedema in the treated skin areas of the three rabbits. One animal showed scaliness after 7 days. The skin irritation resolved within 14 days after exposure for the first animal and 48 hours after exposure for the other two animals.

Based on these results MLA-3202 does not have to be classified and has no obligatory labelling requirement for skin irritation according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) of the United Nations (2015) (including all amendments) and Regulation (EC) No 1272/2008 on classification, labelling and packaging of items and mixtures (including all amendments).

## 4. INTRODUCTION

Due to the acquisition of WIL Research by Charles River, the name of the WIL Research facility in Den Bosch, has been changed to Charles River Laboratories Den Bosch BV, Hambakenwetering 7, 5231 DD 's-Hertogenbosch, The Netherlands. Study documents may contain both names and both names are considered equivalent and may be used as the name of WIL Research transitions to Charles River.

### 4.1. Study Schedule

Experimental starting date : 10 June 2016

Experimental completion date : 11 July 2016

### 4.2. Purpose

The purpose of this primary skin irritation study was to assess the possible irritation or corrosion potential of a single dose of the test item when administered to the intact skin of rabbits. This study should provide a rational basis for risk assessment in man. The absence of skin pigmentation in the albino rabbit facilitates the evaluation of induced skin reactions. The dermal route was selected because the test item may accidentally come into contact with the skin during manufacture, handling and/or use.

### 4.3. Guidelines

This type of study plan was reviewed and agreed by the Laboratory Animal Welfare Officer and the Ethical Committee (DEC 14-21) as required by the Dutch Act on Animal Experimentation (February 1997).

The study procedures described in this report were in compliance with the following guidelines:

- Organisation for Economic Co-operation and Development (OECD), OECD Guidelines for Testing of Chemicals, Section 4, Health Effects, No.404: "Acute Dermal Irritation / Corrosion", Paris, 2015.
- Commission Regulation (EC) No 440/2008 Part B: Methods for the Determination of Toxicity and other Health Effects; B4: "Acute Toxicity: Dermal Irritation/Corrosion". Official Journal of the European Union No. L142, May 2008, including most recent amendments.
- United States Environmental Protection Agency (EPA). Health Effects Test Guidelines, OPPTS 870.2500, Acute Dermal Irritation. Office of Prevention, Pesticides and Toxic Items (7101), EPA 712-C-98-196, August 1998.
- Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF), 12 Nousan, Notification No 8147, November 2000; including the most recent partial revisions.

#### 4.4. Retention of Records and Materials

Records and material pertaining to the study, which include study plan and amendments, raw data, specimens, except perishable specimens, and the final report will be retained in the archives of the test facility for a minimum of 5 years after the finalization of the report. After this period, the sponsor will be contacted to determine how the records and materials should be handled. The test facility will retain information concerning decisions made.

A sample of the test item will be retained until expiry date or applicable retest date. After this period the sample(s) will be destroyed.

#### 4.5. Responsible Personnel

##### 4.5.1. Test Facility

Study Director	A.H.B.M. van Huygevoort, MSc.
Coordinating Biotechnician	R. Eyndhoven (Charles River Den Bosch)
QA	C.J. Mitchell, BSc. (Charles River Den Bosch): <a href="mailto:christine.mitchell@crl.com">christine.mitchell@crl.com</a>
Test Facility Management Representative	H.H. Emmen, MSc. (Charles River Den Bosch): <a href="mailto:harry.emmen@crl.com">harry.emmen@crl.com</a>

##### 4.5.2. Sponsor Representative

Study Monitor	Audrey Batoon, PhD.
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**5. MATERIALS AND METHODS****5.1. Test Item****5.1.1. Test Item Information**

Identification	MLA-3202
Appearance	Clear amber-red liquid
Batch	RC-1045
Purity/Composition	UVCB
Test item storage	At room temperature
Stable under storage conditions until	17 February 2019 (expiry date)

See [APPENDIX 2](#); Certificate of analysis.

**5.1.2. Study Specific Test Item Information**

Test item	207258/A
Purity/composition correction factor	No correction factor required
Test item handling	No specific handling conditions required
Stability at higher temperatures	Stable
pH	6-7
Specific gravity/density	0.9394

**5.1.3. Test Item Preparation**

The test item was applied undiluted as delivered by the Sponsor.

No corrections were made for the purity/composition of the test item, since the guidelines require a fixed amount that has to be applied.

**5.2. Test System**

Species	Albino rabbit, New Zealand White, (SPF-Quality). Recognized by international guidelines as the recommended test system (e.g. EC, OECD) Source: Charles River France, L'Arbresle Cedex, France
Number of animals	3 Males.
Age and body weight	At start of dosing, the animals were between 12 and 24 weeks old and body weights were at least 1.5 kg.
Identification	Earmark.
Health inspection	At least prior to dosing. It was ensured that the animals were healthy and that the skin to be treated was intact and free from any abnormality.

### 5.3. Animal Husbandry

#### Conditions

Environmental controls for the animal room were set to maintain 18 to 24°C, a relative humidity of 40 to 70%, at least 10 air changes/hour, and a 12-hour light/12-hour dark cycle. Any variations to these conditions were maintained in the raw data and had no effect on the outcome of the study.

#### Accommodation

Animals were individually housed in labeled cages with perforated floors (Ebeco, Germany, dimensions 67 x 62 x 55 cm) and shelters (Ebeco, Germany, dimensions 40 x 32 x 23 cm).

Acclimatization period was at least 5 days before start of treatment under laboratory conditions.

#### Diet

Pelleted diet for rabbits (Global Diet 2030 from Harlan Teklad®, Mucedola, Milanese, Italy) approximately 100 grams per day. Hay (TecniLab-BMI BV, Someren, The Netherlands) and wooden sticks (Swedish aspen wood, Bioservices, Uden, The Netherlands) were available during the study period.

#### Water

Free access to tap water.

Diet, water, bedding and cage enrichment evaluation for contaminants and/or nutrients was performed according to facility standard procedures. There were no findings that could interfere with the study.

### 5.4. Weight of Evidence Analysis

In the interest of animal welfare and to minimize any testing likely to produce severe responses in animals, a weight of evidence analysis was performed, prior to the start of this *in vivo* skin irritation study in the rabbit. As recommended in the test guidelines, all available information was evaluated (e.g. existing human and animal data, literature, item data supplied by the Sponsor, analysis of structure activity relationships (SAR), physicochemical properties and reactivity (pH, buffering capacity) and *in vitro*, *ex-vivo* and *in vivo* tests) to determine the need for *in vivo* skin testing. It was concluded that there is need to perform this *in vivo* skin irritation study in rabbit in order to establish the possible skin irritating properties of the test item.

### 5.5. Study Design

The study was performed in a stepwise manner and was started by treatment of a single rabbit (sentinel). The two other animals were treated in a similar manner 19 days later, after considering the degree of skin irritation observed in the first animal.

**5.6. Treatment**

Approximately 24 hours before treatment, the dorsal fur was clipped with electric clippers, exposing an area of approximately 150 square centimetres (10x15 cm).

Each animal was treated by dermal application of 0.5 mL of the test item. The test item was applied to the skin of one flank, using a metalline patch<sup>#</sup> of 2x3 cm. The patch was mounted on Micropore tape<sup>#</sup>, which was wrapped around the abdomen and secured with Coban elastic bandage<sup>#</sup>. Four hours after the application, the dressing was removed and the skin cleaned of residual test item using tap water.

<sup>#</sup>. Suppliers: Lohmann & Rauscher B.V., Almere, The Netherlands (Metalline) and 3M, St. Paul, Minnesota, U.S.A. (Micropore and Coban).

After the final observation, the animals were sacrificed by intra-venous injection of Euthasol<sup>®</sup> 20% (AST Farma BV, Oudewater, The Netherlands).

**5.7. Observations**

Mortality/Viability	Twice daily.
Toxicity	At least once daily.
Body Weight	Day of treatment (prior to application) and on the day of the final observation.
Irritation	The skin reactions were assessed at approximately 1, 24, 48 and 72 hours and 7 and 14 days after the removal of the dressings and test item for the first animal and at approximately 1, 24, 48 and 72 hours after the removal of the dressings and test item for the other two animals. The irritation scores and a description of all other (local) effects were recorded. Adjacent areas of the untreated skin of each animal served as controls.

The irritation was assessed according to the following numerical scoring system. At each observation, the highest scores given were recorded:

*Erythema and eschar formation:*

No erythema.....	0
Very slight erythema (barely perceptible).....	1
Well-defined erythema.....	2
Moderate to severe erythema .....	3
Severe erythema (beef redness) *.....	4

\*. Where signs of necrosis or corrosion (injuries in depth) prevent erythema scoring, the maximum grade for erythema (= 4) is given.

*Oedema formation:*

No oedema .....	0
Very slight oedema (barely perceptible).....	1
Slight oedema (edges of area well-defined by definite raising).....	2
Moderate oedema (raised approximately 1 millimeter) .....	3
Severe oedema (raised more than 1 millimeter and extending beyond the area of exposure).....	4

Necropsy No necropsy was performed according to study plan.

**5.8. Histopathology**

No histopathology was performed.

## **5.9. Interpretation**

The results were evaluated according to:

- Globally Harmonized System of Classification and Labelling of Chemicals (GHS) of the United Nations (2015) (including all amendments).
- Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of items and mixtures (including all amendments).

## **5.10. List of Deviations**

### **5.10.1. List of Study Plan Deviations**

1. Deviations from the maximum level of daily mean relative humidity occurred.  
Evaluation: Laboratory historical data do not indicate an effect of the deviations.
2. The test item was dosed undiluted as delivered by the Sponsor.  
Evaluation: The study plan inadvertently indicated the procedure for dosing solids. The correct procedure for liquids was followed.
3. One animal was also weighed prior to the end of the observation period.  
Evaluation: This additional weighing did not affect the purpose of the study.

The study integrity was not adversely affected by the deviation.

### **5.10.2. List of Standard Operating Procedures Deviations**

Any deviations from standard operating procedures were evaluated and filed in the study file. There were no deviations from standard operating procedures that affected the integrity of the study.

## **6. ELECTRONIC SYSTEMS FOR DATA ACQUISITION**

The following electronic system was used for data acquisition:

- REES Centron Environmental Monitoring system version SQL 2.0 (REES scientific, Trenton, NJ, USA).

## 7. RESULTS

For detailed results see [APPENDIX 1](#).

### 7.1. Irritation

Four hours exposure to 0.5 mL of MLA-3202 resulted in very slight to well-defined erythema and very slight oedema in the treated skin areas of the three rabbits. One animal showed scaliness after 7 days. The skin irritation resolved within 14 days after exposure for the first animal and 48 hours after exposure for the other two animals.

### 7.2. Corrosion

There was no evidence of a corrosive effect on the skin.

### 7.3. Coloration / Remnants

No staining of the treated skin by the test item was observed and no test item remnants were seen.

### 7.4. Toxicity / Mortality

No signs of systemic toxicity were observed in the animals during the test period and no mortality occurred.

## 8. CONCLUSION

Based on these results MLA-3202 does not have to be classified and has no obligatory labelling requirement for skin irritation according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) of the United Nations (2015) (including all amendments) and Regulation (EC) No 1272/2008 on classification, labelling and packaging of items and mixtures (including all amendments).

**APPENDIX 1**  
**TABLES**

**Table 1**  
**Individual Skin Irritation Scores**

Animal	106 <sup>1</sup>			116			117		
	Erythema (0-4)	Oedema (0-4)	comments	Erythema (0-4)	Oedema (0-4)	comments	Erythema (0-4)	Oedema (0-4)	comments
Time after exposure									
1 hour	1	1	-	0	1	-	1	1	-
24 hours	2	1	-	0	1	-	0	1	-
48 hours	1	0	-	0	0	-	0	0	-
72 hours	1	0	-	0	0	-	0	0	-
7 days	0	0	a	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
14 days	0	0	-	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

<sup>1</sup> Sentinel.

Comments:

- No clinical signs observed

n.a. Not applicable, animal sacrificed after 72 hours according to study plan.

a. Scaliness.

**Table 2**  
**Mean Value Irritation Scores**

Animal	Mean 24, 48 and 72 hrs	
	Erythem	Oedema
106	1.3	0.3
116	0.0	0.3
117	0.0	0.3

**Table 3**  
**Animal Specifications**

Animal	Sex	Age at start (weeks)	Body weights (grams)	
			prior to application	after the final observation
106 <sup>1</sup>	♂	12	2341	2728
116	♂	13	2846	2941
117	♂	13	3070	3208

1. Animal was inadvertently also weighed on Day 8 (2425 grams)

**APPENDIX 2**  
**TEST ITEM CERTIFICATE OF ANALYSIS**



Chemtura Corporation  
12 Spencer St  
Naugatuck, CT 06770

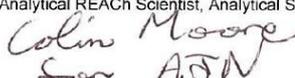
Analytical Services  
[www.chemtura.com](http://www.chemtura.com)

### Certificate of Purity

Customer: Support for Toxicology Studies  
Test Substance Name: MLA3202; Amides, tallow, N,N-bis(2-hydroxypropyl)  
Physical Appearance: Liquid  
CAS No.: 1454803-04-3  
Ref. or Lot Number: RC-1045  
Date of Analysis: revised March 18, 2016 (original issue March 7, 2016)

Percent Composition	Monoisotopic Mass (daltons)	Formula	Structure/ Identity
33.1	397.4	C <sub>24</sub> H <sub>47</sub> NO <sub>3</sub>	C18:1 (oleic) tallow amides, N,N-bis(2-hydroxypropyl)
22.9	371.3	C <sub>22</sub> H <sub>45</sub> NO <sub>3</sub>	C16:0 (palmitic) tallow amides, N,N-bis(2-hydroxypropyl)
13.6	395.4	C <sub>24</sub> H <sub>45</sub> NO <sub>3</sub>	C18:2 (linoleic) tallow amides, N,N-bis(2-hydroxypropyl)
11.0	399.4	C <sub>24</sub> H <sub>49</sub> NO <sub>3</sub>	C18:0 (stearic) tallow amides, N,N-bis(2-hydroxypropyl)
6.0	369.3	C <sub>22</sub> H <sub>43</sub> NO <sub>3</sub>	C16:1 (palmitoleic) tallow amides, N,N-bis(2-hydroxypropyl)
3.2	419.3	C <sub>26</sub> H <sub>45</sub> NO <sub>3</sub>	C20:4 (eicosatetraenoic) tallow amides, N,N-bis(2-hydroxypropyl)
2.0	393.3	C <sub>24</sub> H <sub>43</sub> NO <sub>3</sub>	C18:3 (linolenic) tallow amides, N,N-bis(2-hydroxypropyl)
1.5	282.3	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	C18:1 (oleic) acid
1.1	421.4	C <sub>26</sub> H <sub>47</sub> NO <sub>3</sub>	C20:3 (eicosatrienoic) tallow amides, N,N-bis(2-hydroxypropyl)
5.6			Sum of residual components (< 1% each)
100.0			Total

  
 Blake Lewis  
 Analytical REACH Scientist, Analytical Services  
 Date 3/7/16

  
 Colin Moore  
 Sr. Technology Manager  
 Analytical and Lab Support Services  
 Date 3/7/16

**APPENDIX 3**  
**ENDORSEMENT OF COMPLIANCE WITH THE OECD PRINCIPLES OF GLP**



## ENDORSEMENT OF COMPLIANCE

### WITH THE OECD PRINCIPLES OF GOOD LABORATORY PRACTICE

Pursuant to the Netherlands GLP Compliance Monitoring Programme and according to Directive 2004/9/EC the conformity with the OECD Principles of GLP was assessed on 7 – 11, 14 and 16 September 2015 at

WIL Research Europe B.V.  
Hambakenwetering 7  
5231 DD 's Hertogenbosch

It is herewith confirmed that the afore-mentioned test facility is currently operating in compliance with the OECD Principles of Good Laboratory Practice in the following area of expertise: physical-chemical testing, toxicity studies, mutagenicity studies, environmental toxicity studies on aquatic and terrestrial organisms, studies on behaviour in water, soil, and air, bioaccumulation, residue studies, analytical and clinical chemistry testing, kinetic and metabolism studies and safety pharmacology.

Utrecht, 3 November 2015

Dr R.M.A. Jaspers  
Coordinating/specialist senior inspector